for claim 30 can be found at page 12, line 14. Support for claims 31 and 32 can be found at Figures 1 and 2, at page 12, lines 13-14; and at page 6, lines 21-34, which states that all eight cysteines and the signature motif for the PDGF/VEGF family is conserved in VEGF3 and calls attention to Figure 2. From looking at Figure 2, one of ordinary skill in the art would appreciate that amino acids 69 to 82 of SEQ ID NO:2 contain the signature motif, and that the portion of VEGF3 that contains the conserved cysteines and motif includes amino acids 46 to 123 of SEQ ID NO:2.

Support for claims 34-35 can be found at page 10, lines 28-33; at page 12, line 14; and at page 29, line 27 to page 30, line 5. Support for claims 36-37 can be found at page 10, lines 28-33; at page 12, line 14; and at page 18, lines 32-33. Support for claims 42-44 and 46-49 can be found at page 7, lines 21-24; and at page 8, lines 16-26. Support for claims 52-56 can be found at page 18, lines 21-27. Support for claims 61-65 can be found at page 11, line 32 to page 12, line 2; and at page 24, line 4 to page 25, line 4.

Applicants point out that the filing date of U.S. Patent No. 5,928,939 is December 6, 1995, which is after the filing date of the present priority application. Additionally, U.S. Patent No. 5,928,939 is a continuation-in-part of U.S. Appl. No. 08/469,427, filed June 6, 1995, which is a continuation-in-part of U.S. Appl. No. 08/397,651, filed March 1, 1995. U.S. Appl. No. 08/469,427 was filed the same day as the present priority application. Thus, only the content of U.S. Appl. No. 08/397,651 may potentially be available as prior art against the pending claims.

Applicants note that 35 U.S.C. § 102(e) refers to description in a patent application granted or an application for patent by another filed in the United States *before* the invention thereof by the applicant for patent. The captioned application is a divisional of U.S. App. No. 08/469,641, filed June 6, 1995. Since Appl. No. 08/397,651 was filed the same day as the priority application, Applicants assert that its contents cannot constitute prior art to this application as a matter of law.

Nevertheless, in an effort to expedite prosecution, Applicants enclose herewith a copy of U.S. Appl. No. 08/397,651. The Examiner states that "Eriksson et al. teach a polypeptides [sic] of SEQ ID NO:11 and 15 which share a contiguous stretch of 119 and 149 identical amino acids,

respectively, with the polypeptide of SEQ ID NO:2 of the instant application." Paper No. 14, page 3. SEQ ID NOs:11 and 15 of Eriksson *et al.* are not disclosed in U.S. Appl. No. 08/397,651. Accordingly, even if it would be assumed *arguendo* that the content of U.S. Appl. No. 08/397,651 would be available as prior art, the present claims would still not be anticipated by Eriksson *et al.* Withdrawal of this rejection is respectfully requested.

Conclusion

All of the stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot. Applicants therefore respectfully request that the Examiner reconsider all presently outstanding objections and rejections and that they be withdrawn. Applicants believe that a full and complete reply has been made to the outstanding Office Action and, as such, the present application is in condition for allowance. If the Examiner believes, for any reason, that personal communication will expedite prosecution of this application, the Examiner is invited to telephone the undersigned at the number provided.

Prompt and favorable consideration of this Amendment and Reply is respectfully requested.

Respectfully submitted,

STERNE, KESSLER, GOLDSTEIN & FOX P.L.L.C.

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CONTINUING DATA********
VERIFIED

_____

FOREIGN/PCT APPLICATIONS********
VERIFIED

_____

STATE OR COUNTRY	SHEETS DRAWING	TOTAL CLAIMS	INDEPENDENT CLAIMS	FILING FEE RECEIVED	ATTORNEY DOCKET NO.
SEX	3	23	2	$926.00	1064/41979

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TITLE

VASCULAR ENDOTHELIAL GROWTH FACTOR-B AND DNA CODING THEREFOR

This is to certify that annexed hereto is a true copy from the records of the United States Patent and Trademark Office of the application which is identified above.

By authority of the
COMMISSIONER OF PATENTS AND TRADEMARKS

Date APR 18 1996 Certifying Officer

08 397651

Inventors: Ulf Eriksson
Birgitta Olofsson

VASCULAR ENDOTHELIAL GROWTH FACTOR-B AND DNA CODING THEREFOR

Background of the Invention

Angiogenesis, or the proliferation of new capillary blood vessels, is a fundamental process necessary for normal growth and development of tissues. It is a prerequisite for the development and differentiation of the vascular tree, as well as for a wide variety of fundamental physiological processes including embryogenesis, somatic growth, tissue and organ repair and regeneration, cyclical growth of the corpus luteum and endometrium, and development and differentiation of the nervous system. In the female reproductive system, angiogenesis occurs in the follicle during its development, in the corpus luteum following ovulation and in the placenta to establish and maintain pregnancy. Angiogenesis additionally occurs as part of the body's repair processes, e.g. in the healing of wounds and fractures. Angiogenesis is also a factor in tumor growth, since a tumor must continuously stimulate growth of new capillary blood vessels in order to grow.

Capillary blood vessels consist of endothelial cells and pericytes. These two cell types carry all of the genetic information to form tubes, branches and entire capillary networks. Specific angiogenic molecules can initiate this process. In view of the physiological importance of angiogenesis, much effort has been devoted to the isolation, characterization and purification of factors that can stimulate angiogenesis, and a number of polypeptides which stimulate angiogenesis have been purified and characterized as to their molecular, biochemical and

biological properties. For reviews of such angiogenesis regulators, see Klagsbrun et al., "Regulators of Angiogenesis", *Ann. Rev. Physiol.*, 53:217-39 (1991); and Folkman et al., "Angiogenesis," *J. Biol. Chem.*, 267:10931-934 (1992).

One such growth factor, which is highly specific as a mitogen for vascular endothelial cells, is termed vascular endothelial growth factor (VEGF). See Ferrara et al., "The Vascular Endothelial Growth Factor Family of Polypeptides," *J. Cellular Biochem.*, 47:211-218 (1991); Connolly, "Vascular Permeability Factor: A Unique Regulator of Blood Vessel Function," *J. Cellular Biochem.*, 47:219-223 (1991). VEGF is a potent vasoactive protein that has been detected in media conditioned by a number of cell lines including bovine pituitary follicular cells. VEGF is a glycosylated cationic 46-48 kD dimer made up of two 24 kD subunits. It is inactivated by sulfhydryl reducing agents, resistant to acidic pH and to heating, and binds to immobilized heparin. VEGF is sometimes referred to as vascular permeability factor (VPF) because it increases fluid leakage from blood vessels following intradermal injection. It also has been called by the name vasculotropin.

Four different molecular species of VEGF have been detected. The 165 amino acid species has a molecular weight of approximately 46 kD and is the predominant molecular form found in normal cells and tissues. A less abundant, shorter form with a deletion of 44 amino acids between positions 116 and 159 ($VEGF_{121}$), a longer form with an insertion of 24 highly basic residues in position 116 ($VEGF_{189}$), and another longer form with an insertion of 41 amino acids ($VEGF_{206}$), which includes the 24 amino acid insertion found in $VEGF_{189}$, are also known. $VEGF_{121}$ and $VEGF_{165}$ are soluble proteins. $VEGF_{189}$ and $VEGF_{206}$ appear to be mostly cell-associated. All of the versions of VGEF are biologically active. For example, each of the species when applied intradermally is able to induce extravasation of Evans blue.

The various species of VEGF are encoded by the same gene and arise from alternative splicing of messenger RNA. This conclusion is supported by Southern blot analysis of human genomic DNA, which shows that the restriction pattern is identical using either a probe for $VEGF_{165}$ or one which contains the insertion in $VEGF_{206}$. Analysis of genomic clones in the area of putative mRNA splicing also shows an intron/exon structure consistent with alternative splicing.

Analysis of the nucleotide sequence of the VEGF gene indicates that VEGF is a member of the platelet-derived growth factor (PDGF) family. The amino acid sequence of VEGF exhibits approximately 20% homology to the sequences of the A and B chains of PDGF, as well as complete conservation of the eight cysteine residues found in both mature PDGF chains. VEGF also contains eight additional cysteine residues within the carboxy-terminal region. The amino-terminal sequence of VEGF is preceded by 26 amino acids corresponding to a typical signal sequence. The mature protein is generated directly following signal sequence cleavage without any intervening prosequence. The existence of a potential glycosylation site at Asn^{74} is consistent with other evidence that VEGF is a glycoprotein, but the polypeptide has been reported to exist in both glycosylated and deglycosylated species.

Like other cytokines, VEGF can have diverse effects that depend on the specific biological context in which it is found. VEGF is a potent endothelial cell mitogen and directly contributes to induction of angiogenesis *in vivo* by promoting endothelial cell growth during normal development or during wound healing. A most striking property of VEGF is its specificity. It is mitogenic *in vitro* at 1 ng/ml for capillary and human umbilical vein endothelial cells, but not for adrenal cortex cells, corneal or lens epithelial cells, vascular smooth muscle cells, corneal endothelial cells, granulosa cells, keratinocytes, BHK-21 fibroblasts, 3T3 cells, rat embryo fibroblasts, human placental

fibroblasts and human sarcoma cells. The target cell specificity of VEGF is thus restricted to vascular endothelial cells. VEGF can trigger the entire sequence of events leading to angiogenesis and stimulates angiogenesis *in vivo* in the cornea and in a healing bone graft model. It is able to stimulate the proliferation of endothelial cells isolated from both small and large vessels. Expression of VEGF mRNA is temporally and spatially related to the physiological proliferation of capillary blood vessels in the ovarian corpus luteum or in the developing brain. VEGF expression is triggered by hypoxemia so that endothelial cell proliferation and angiogenesis appear to be especially stimulated in ischemic areas. VEGF is also a potent chemoattractant for monocytes. In addition, VEGF induces plasminogen activator and plasminogen activator inhibitor in endothelial cells.

Tumor cells release angiogenic molecules such as VEGF, and monoclonal antibodies to VEGF have been shown to inhibit the growth of certain types of tumor such as rhabdomyosarcoma. See Kim et al., "Inhibition of Vascular Endothelial Growth Factor-Induced Angiogenesis Suppresses Tumor Growth *in vivo*," *Nature*, 362:841-844 (1993). This suggests that blocking VEGF action is of potential therapeutic significance in treating tumors in general, and highly-vascularized, aggressive tumors in particular.

Summary of the Invention

It is an object of the invention to provide a new growth factor having the property of promoting proliferation of endothelial cells.

Another object of the invention is to provide isolated DNA sequences which encode a new growth factor which promotes proliferation of endothelial cells.

It is also an object of the invention to provide new products which may be useful in diagnostic and/or therapeutic applications.

These and other objects are achieved in accordance with the present invention by providing an isolated DNA sequence which codes for a protein having the property of promoting proliferation of endothelial cells or mesodermal cells, the DNA sequence hybridizing under stringent conditions with a coding portion of the DNA sequence of Figure 1 or Figure 2.

In accordance with further aspects of the invention, the objects are also achieved by providing a mammalian protein having the property of promoting proliferation of endothelial cells, which protein comprises an amino acid sequence substantially corresponding to the amino acid sequence of Figure 1 or the amino acid sequence of Figure 2, and by providing pharmaceutical preparations which comprise such proteins and antibodies which react with such proteins.

Clinical applications of the invention include diagnostic applications, acceleration of angiogenesis in wound healing, and inhibition of angiogenesis. Quantitation of VEGF-B in cancer biopsy specimens may be useful as an indicator of future metastatic risk. Topical application of VEGF-B preparations to chronic wounds may accelerate angiogenesis and wound healing.

Brief Description of the Drawings

Figure 1 shows the nucleotide sequence of the (partial) cDNA clone of VEGF-B and the amino acid sequence of the protein segment coded by the first reading frame of the cDNA;

Figure 2 repeats the nucleotide sequence of the (partial) cDNA clone of VEGF-B and the amino acid sequence of the protein segment coded by the second reading frame of the cDNA; and

Figure 3 shows a comparison of the amino acid sequences of PDGF-A, PDGF-B, PlGF, VEGF and VEGF-B.

Detailed Description of Preferred Embodiments

The present invention thus is directed to a new vascular endothelial growth factor, hereinafter referred to as VEGF-B, which shares the angiogenic and other properties of VEGF, but which is distributed and expressed in tissues differently from VEGF.

VEGF-B is a member of the family of platelet derived growth factors and is a growth factor which promotes mitosis and proliferation of vascular endothelial cells and/or mesodermal cells. It is produced by expression of a DNA sequence which is hybridizable under stringent conditions with the DNA sequence depicted in Figure 1. Suitable hybridization conditions include, for example, 50% formamide, 5 x SSPE buffer, 5 x Denhardts solution, 0.5% SDS and 100 μg/ml of salmon sperm DNA at 42°C overnight, followed by washing 2 x 30 minutes in 2 x SSC at 55°C.

The invention is also directed to an isolated and/or purified DNA which hybridizes under stringent conditions with the DNA sequence of Figure 1 or Figure 2. It is intended to include within the scope of the invention all angiogenic proteins encoded by DNA sequences which hybridize under stringent conditions to the DNA sequence of Figures 1 and 2.

In a further aspect, the invention is directed to antibodies of VEGF-B, and particularly to monoclonal antibodies.

A cDNA clone encoding murine VEGF-B was identified as follows. A cDNA library (E 14.5) derived from poly A+ mRNA isolated from 14.5 day old mouse embryos [Chevray P. and Nathans D., "Protein interaction cloning in yeast: identification of mammalian proteins that react with the leucine zipper of Jun," *Proc. Natl. Acad. Sci. USA*, 89:5789-93 1992)] was screened for cellular proteins which potentially might interact with cellular retinoic acid-binding protein type 1 (CRABP-I) using a yeast two-hybrid interaction trap screening technique as described by Gyuris

J., Golemis E., Chertkov H. and Brent R., "Cdi1, a Human G1 and S Phase Protein Phosphatase That Associates with Cdk2," *Cell*, 75:791-803 (1993). This screening technique involves a fusion protein that contains a binding domain and that is known to be transcriptionally inert (the "bait"); reporter genes that have no basal transcription and that are bound by the bait; and an expression library which encodes proteins expressed as chimeras and whose amino termini contain an activation domain and other useful moieties (the "prey"). The screened library was a plasmid library in the yeast expression vector pPC67 obtained from Dr. Pierre Chevray of the Johns Hopkins University, School of Medicine, 725 North Wolfe St., Baltimore, MD 21205. A positive cDNA clone (pcif-2) was recovered from the screening. The positive clone was sequenced using well known, conventional techniques and found to encode a protein highly homologous to VEGF and the other members of the PDGF family of growth factors. The 890 base pair SalI-NotI insert in the plasmid pPC67 was cloned into pBluescript and fully sequenced using T7 and T3 vector primers together with internal primers. The plasmid pBluescript is commercially available from Stratagene Inc., LaJolla, California. The cDNA insert was found to be 886 base pairs long and to encode two polypeptides in different reading frames which were homologous to the N-terminal end and the C-terminal end, respectively, of VEGF. This novel growth factor is referred to hereinafter as VEGF-B. The clone is partial and lacks approximately seven amino acids in the amino terminal region and the entire signal sequence of approximately twenty-eight amino acids.

The protein is believed to interact with protein tyrosine kinase growth factor receptors. Details of such receptors are known in the art (See e.g. Wilks, A.F., "Protein Tyrosine Kinase Growth Factor Receptors and Their Ligands in Development, Differentiation, and Cancer," *Adv. Cancer Res.*, 60:43-73 (1993)).

Various adult mouse tissues were tested for expression of transcripts corresponding to VEGF-B by Northern blotting. The size of the mRNA was 1.3-1.4 kb. A mouse multiple tissue Northern blot (MTN, Clontech) was probed with the 0.89 kb *Sal*1-*Not*1 fragment derived from the pPC67 yeast expression vectors described above. The probe was labelled with ^{32}P-dCTP using random priming (specific activity 10^8-10^9 cpm/µg of DNA). The blot was hybridized overnight at 42°C using 50% formamide, 5 x SSPE buffer, 2% SDS, 10 x Denhardts solution, 100 µg/ml salmon sperm DNA and $1x10^6$ cpm of the labelled probe/ml. The blot was washed at room temperature for 2 x 30 min in 2 x SSC containing 0.05% SDS and then for 2 x 20 min at 52°C in 0.1 x SSC containing 0.1% SDS. The blot was then exposed at -70°C for three days using intensifying screens. Kodak XAR film was used. The relative expression levels as determined by visual examinations of the film are listed in the following table:

Table 1

Distribution of VEGF-B Transcripts in the Adult Mouse

Tissue	Relative Expression Level
Heart	+++++
Brain	+++
Spleen	(+)
Lung	++
Liver	+
Skeletal Muscle	++++
Kidney	+++
Testis	(+)

+++++ = very strong expression; ++ = rather weak expression;
++++ = strong expression; + = weak expression;
+++ = moderate expression; (+) = very weak expression.

A human multiple tissue Northern blot (MNT) from Clontech was probed using the murine partial cDNA to determine relative VEGF-B expression levels in various human tissues. The size of the transcript was 1.3-1.4 kb. The conditions were identical to those used for the mouse Northern blot described above. The relative VEGF-B transcript levels for the human Northern blot are listed in the following Table 2. For comparison purposes, Table 2 also lists relative expression level data from the literature for VEGF in various mammalian systems.

Table 2

Tissues	Relative Expression Levels			
	VEGF-B (Northern blot)	VEGF (from literature)		
	human	human	murine	guinea pig
heart	+++++	++	+++	+++
brain	+		+	+
placenta	+			
lung	+	++++		++
liver	(+)	++	(+)	+
skeletal muscle	++++		+++	+
kidney	+	++	+	++
pancreas	+++			
spleen	++		-	+
thymus	+		-	
prostate	+++			
testis	++			(+)
ovary	+++			-
small intestine	++			
colon	+++			
peripheral blood leukocytes	+			

From a comparison of Table 1 and Table 2 it can be seen that mouse and human tissue expression levels of VEGF-B transcripts are relatively similar with the highest expression levels being found in heart and skeletal muscle. Significant differences may be seen in brain and kidney tissue. It should also be noted that tissues containing a large proportion of epithelial cells, such as prostate, pancreas and colon from which some of the most common human tumors originate, express relatively high levels of VEGF-B.

A comparison of the relative expression levels of VEGF and VEGF-B in human tissues shows some striking differences. VEGF is expressed rather weakly by human heart tissue, but VEGF-B is very strongly expressed by the same tissue. On the other hand, VEGF is strongly expressed by human lung tissue, but VEGF-B is only weakly expressed by human lung tissue. In a similar vein, human liver tissue expresses VEGF at a moderate level, but VEGF-B is expressed only very weakly. These data evidence that despite their general similarities, the actions of VEGF and VEGF-B are not completely identical.

Figure 1 shows the nucleotide sequence (SEQ ID NO:1) of the partial cDNA clone of VEGF-B and the amino acid sequence (SEQ ID NO:2) encoded in the first reading frame thereof. The DNA sequence of Figure 1 was obtained by conventional sequencing of a clone (pcif-2) in the yeast expression vector pPC67. The clone comprised 886 base pairs and encoded a part of murine VEGF-B.

The isolated cDNA sequence will hybridize with the mammalian genomic DNA, e.g. either murine or human, which contains the VEGF-B gene. In addition to the coding sequence, the genomic DNA will contain one or more promoter sequence(s) which give and direct expression of VEGF-B in one or more specific tissues. Thus the coding sequence of VEGF-B may be linked to an endothelial specific promoter which is specific to a certain type or types of tissue.

The full length protein is estimated to be approximately 120-125 amino acids in length.

The nucleotide sequence is translated in two different reading frames into two different amino acid sequences. There is a stop codon (TGA) within the coding sequence at base pairs 309-311. Thus, VEGF-B comes in several splicing variants. The 5'end of the cloned cDNA sequence encodes an 102 amino acid long protein with significant homology to the N-terminal domains of VEGF, PlGF and PDGF A and B. In particular, a number of cysteine residues are perfectly conserved within this group of proteins. In addition to the nucleotide sequence (SEQ ID NO:1), Figure 1 further depicts the deduced amino acid sequence (SEQ ID NO:2) of this first protein, which is 102 amino acids in length.

Translation of the C-terminal end of the cDNA (base pairs 308-475) in a different reading frame results in a protein which is highly homologous to the C-terminal part of VEGF. Figure 2 again shows the nucleotide sequence (SEQ ID NO:1) of Figure 1, but this time includes the deduced amino acid sequece (SEQ ID NO:3) of the second protein, which is encoded in the second reading frame and is 54 amino acids long. It thus appears that the VEGF-B gene encodes different proteins using alternative splicing of the primary transcript. The last part of the clone, encoding the second peptide might be expressed as a functional protein in other spliced variants of VEGF-B.

Figure 3 shows a comparison of the amino acid sequence alignments of Platelet Derived Growth Factor A (PDGF-A), Platelet Derived Growth Factor B, (PDGF-B), Placenta Growth Factor PlGF, Vascular Endothelial Growth Factor (VEGF) and the novel Vascular Endothelial Growth Factor B of the present invention (VEGF-B). As can be seen from this figure, the homologous relationship of the sequences is apparent, and VEGF-B is a structural homolog of the other growth factors of this group. The boxes in Figure 3

indicate conserved cysteine residues in the respective protein amino acid sequences.

The aforedescribed proteins may exist in combined association with an additional N-terminal sequence of approximately five (5) to ten (10) amino acids, as well as a further leader sequence of approximately twenty-eight (28) amino acids. Inasmuch such combined amino acid sequences exhibit the property of promoting the proliferation of endothelial cells and the DNA sequences which code for such combined peptide sequences will hybridize under stringent conditions with the DNA sequence of Figures 1 and 2, such amino acid sequences and the DNA which codes for them are expressly contemplated to be within the scope of the present invention.

VEGF-B is synthesized normally in the endoplasmic reticulum of the source cell for subsequent export. Recombinant VEGF-B may be produced by inserting a DNA sequence encoding the VEGF-B protein together with a suitable operatively linked promoter and control sequences into a suitable vector, such as the well known plasmid pBR322 or a derivative thereof, transforming or transfecting a suitable host cell, such as a Cos cell, with the resulting vector or other systems well known in the art and screening the resulting transformants for VEGF-B expression, and then culturing cell lines which are positive for the expression of VEGF-B. Either a eukaryotic vector or a prokaryotic vector may be used, depending on the type of cell which is to be transfected or transformed therewith.

VEGF-B can be used as a growth factor for populations of endothelial cells *in vitro*. VEGF-B may be used to promote desirable angiogenesis, i.e. the formation of new blood vessels and capillaries. For example, it may be useful in promoting the development of the corpus luteum and endometrium as an aid to initiating and/or maintaining pregnancy. Administration of VEGF-B may also be useful in supporting embryogenesis, as well as somatic growth and

vascular development and differentiation. Topical application of VEGF-B to wounds may be useful in promoting wound healing, and oral administration of VEGF-B may be useful to accelerate the healing of gastric and/or duodenal ulcers.

VEGF-B may exert proliferative effects on mesodermal cells either directly or via improvements in the blood supply.

Tumor assays for VEGF-B may be useful as indicators of metastatic risk. Assays of VEGF-B in body fluids or the tumor itself by histochemistry may be useful as a tumor prognostic factor. Furthermore, because tumor growth requires angiogenesis, administration of VEGF-B may also be useful in promoting tumor growth in laboratory animals in order to test anti-tumorigenic drugs. VEGF-B may also be useful to increase the microvascularity of hypoxic areas of tumors and make them more sensitive to radiation, radiation sensitizing drugs, etc.

The angiogenic action of VEGF-B may be useful in treating ischemic conditions. VEGF-B or agonists could be used to stimulate the development of collateral circulation in cases of arterial and/or venous obstruction, e.g. myocardial infarcts, ischaemic limbs, deep venous thrombisis, and/or postpartum vascular problems.

A VEGF-B/VEGF-B receptor system may be used as an assay system to detect small molecules as agonists/antagonists for development as new drugs.

Pharmaceutical compositions may be produced by admixing a pharmaceutically effective amount of VEGF-B protein with one or more suitable carriers or adjuvants such as water, mineral oil, polyethylene glycol, starch, talcum, lactose, thickeners, stabilizers, suspending agents, etc. Such compositions may be in the form of solutions, suspensions, tablets, capsules, creams, salves, ointments, or other conventional forms.

VEGF-B protein also can be used to produce antibodies. Such antibodies may be produced using conventional antibody production techniques. For example, specific monoclonal antibodies may be produced via immunization of fusion proteins obtained by recombinant DNA expression. Labelled monoclonal antibodies, in particular, should be useful in screening for conditions associated with abnormal levels of VEGF-B in the body. For example, assays of VEGF-B levels in blood or urine may be useful as a tumor marker. These monoclonal antibodies to VEGF-B also may be useful in inhibiting angiogenesis associated with high levels of VEGF-B in the body, e.g. in rapidly proliferating, angiogenesis-dependent tumors in mammals, and thereby may retard the growth of such tumors. Treatment may be effected, e.g., by twice weekly intraperitoneal injection of 10 to 500 μg, preferably 50-100 μg of monoclonal antibody. For the therapy of humans, chiaserization or humanization of such monoclonal antibodies is to be preferred.

VEGF-B antagonists such as antibodies may be useful to inhibit new blood vessels in diabetic retinopathy, psoriasis, arthopathies and/or vascular tumors such as haemangiomas.

The foregoing description and examples have been set forth merely to illustrate the invention and are not intended to be limiting. Since modifications of the disclosed embodiments incorporating the spirit and substance of the invention may occur to persons skilled in the art, the invention should be construed to include everything within the scope of the appended claims and equivalents thereof.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

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(ii) TITLE OF INVENTION: VASCULAR ENDOTHELIAL GROWTH FACTOR-B AND DNA CODING THEREFOR

(iii) NUMBER OF SEQUENCES: 3

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

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(viii) ATTORNEY/AGENT INFORMATION:
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(C) REFERENCE/DOCKET NUMBER: 1064/41979

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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 886 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CGGGACGCCC AGTGGTGCCA TGGATAGACG TTTATGCACG TGCCACATGC CAGCCCAGGG 60

AGGTGGTGGT GCCTCTGAGC ATGGAACTCA TGGGCAATGT GGTCAAACAA CTAGTGCCCA 120

GCTGTGTGAC TGTGCAGCGC TGTGGTGGCT GCTGCCCTGA CGATGGCCTG GAATGTGTGC 180

CCACTGGGCA ACACCAAGTC CGAATGCAGA TCCTCATGAT CCAGTACCCG AGCAGTCAGC 240

TGGGGGAGAT GTCCCTGGAA GAACACAGCC AATGTGAATG CAGACCAAAA AAAAAAAGGA 300

GAGTGCTGTG AAGCCAGACA GCCCCAGGAT CCTCTGCCCG CCTTGCACCC AGCGCCGTCA 360

ACGCCCTGAC CCCCGGACCT GCCGCTGCCG CTGCAGACGC CGCCGCTTCC TCCATTGCCA 420

AGGGCGGGGC TTAGAGCTCA ACCCAGACAC CTGTAGGTGC CGGAAGCCGC GAAAGTGACA 480

AGCTGCTTTC CAGACTCCAC TGGCCCGGCT GCTTTTATGG CCCTGCTTCA CAGGGACGAA 540

GAGTGGAGCA CAGGCAAACC TCCTCAGTCT GGGAGGTCAC TGCCCCAGGA CCTGGACCTT 600

TTAGAGAGCT CTCTCGCCAT CTTTTATCTC CCAGAGCTGC CATCTAACAA TTGTCAAGGA 660

ACCTCATGTC TCACCTCAGG GGCCAGGGTA CTCTCTCACT TAACCACCCT GGTCAAGTGA 720

GCATCTTCTG GCTGGCTGTC TCCCCTCACT ATGAAAACCC CAAACTTCTA CCAATAACGG 780

GATTTGGGTT CTGTTATGAT AACTGTGACA CACACACACA CTCACACTCT GATAAAAGAG 840

AACTCTGATA AAAGAGATGG AAGACACTAA AAAAAAAAAA AAAAAA 886

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 102 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Arg Pro Val Val Pro Trp Ile Asp Val Tyr Ala Arg Ala Thr Cys
1 5 10 15

Gln Pro Arg Glu Val Val Val Pro Leu Ser Met Glu Leu Met Gly Asn
20 25 30

Val Val Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly
35 40 45

Gly Cys Cys Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His
50 55 60

Gln Val Arg Met Gln Ile Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu
65 70 75 80

Gly Glu Met Ser Leu Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys
85 90 95

Lys Lys Arg Arg Val Leu
100

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 55 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Lys Pro Asp Ser Pro Arg Ile Leu Cys Pro Pro Cys Thr Gln Arg Arg
1 5 10 15

Gln Arg Pro Asp Pro Arg Thr Cys Arg Cys Arg Cys Arg Arg Arg Arg
20 25 30

Phe Leu His Cys Gln Gly Arg Gly Leu Glu Leu Asn Pro Asp Thr Cys
35 40 45

Arg Cys Arg Lys Pro Arg Lys
50 55

What is claimed is:

1. An isolated DNA sequence which codes for a protein having the property of promoting proliferation of endothelial cells or mesodermal cells, said DNA sequence hybridizing under stringent conditions with a coding portion of the DNA sequence (SEQ ID NO:1) of Figure 1 or Figure 2.

2. A DNA sequence according to claim 1, wherein said DNA sequence is a cDNA sequence.

3. A DNA sequence according to claim 1, comprising a cDNA sequence corresponding to the DNA sequence of Figure 1 or Figure 2.

4. A DNA sequence according to claim 1, wherein said DNA sequence is a mammalian DNA sequence.

5. A DNA sequence according to claim 4, wherein said DNA sequence is a murine DNA sequence.

6. A DNA sequence according to claim 1, wherein said DNA sequence codes for a protein which promotes proliferation of vascular endothelial cells.

7. A vector comprising a DNA sequence according to claim 1.

8. A vector according to claim 7, wherein said vector is a eukaryotic vector.

9. A vector according to claim 7, wherein said vector is a prokaryotic vector.

10. A vector according to claim 7, wherein said vector is a plasmid.

11. A protein having the property of promoting proliferation of endothelial cells or mesodermal cells, said protein comprising an amino acid sequence substantially corresponding to the amino acid sequence of Figure 1 or the amino acid sequence of Figure 2.

12. A protein according to claim 11, wherein said protein comprises an amino acid sequence corresponding to the amino acid sequence (SEQ ID NO:2) of Figure 1.

13. A protein according to claim 11, wherein said protein comprises an amino acid sequence corresponding to the amino acid sequence (SEQ ID NO:3) of Figure 2.

14. A protein according to claim 11, wherein said protein is a mammalian protein.

15. A protein according to claim 14, wherein said protein is a murine protein.

16. A protein according to claim 11, wherein said protein promotes proliferation of vascular endothelial cells.

17. A pharmaceutical composition comprising an effective endothelial or mesodermal cell proliferation promoting amount of a protein according to claim 11, and at least one conventional pharmaceutical carrier or diluent.

18. An antibody which reacts with a protein according to claim 11.

19. An antibody according to claim 18, wherein said antibody is a monoclonal antibody.

20. A host cell transformed or transfected with a vector according to claim 7, such that said host cell expresses a protein having the property of promoting proliferation of endothelial or mesodermal cells.

21. A transformed host cell according to claim 20, wherein said host cell is a eukaroytic cell.

22. A cell according to claim 21, wherein said host cell is a COS cell.

23. A transformed host cell according to claim 20, wherein said host cell is a prokaryotic cell.

Abstract of the Disclosure

Polypeptide, VEGF-B, from the PDGF family of growth factors having the property of promoting mitosis and proliferation of vascular endothelial cells, DNA sequences encoding these polypeptides, pharmaceutical compositions containing them and antibodies which react with them. The VEGF-B polypeptides are useful in stimulating angiogenesis as well as in diagnostic applications.

Attorney Docket No. 1064/41979

DECLARATION AND POWER OF ATTORNEY - PATENT APPLICATION

As a below named inventor, I hereby declare that my citizenship, postal address and residence are as stated below; that I verily believe I am the original, first and sole inventor (if only one inventor is named below) or a joint inventor (if plural inventors are named below) of the invention entitled:

VASCULAR ENDOTHELIAL GROWTH FACTOR-B AND DNA CODING THEREFOR

the specification of which

___ is attached hereto, or

_X_ was filed on March 1, 1995 as Application Serial No. 08/397,651 and

was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose all information known to be material to patentability as defined in 37 CFR §1.56. I hereby claim foreign priority benefits under Title 35, United States Code §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Prior Foreign Application(s)			Priority Claimed
(Number)	(Country)	(Day/Month/Year)	
(Number)	(Country)	(Day/Month/Year)	

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose all information known to be material to patentability as defined in 37 CFR §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

Application Serial No.:	(Filing Date)	(Status)

I hereby appoint as principal attorneys Herbert I. Cantor, Reg. No. 24,392; James F. McKeown, Reg. No. 25,406; Donald D. Evenson, Reg. No. 26,160; Joseph D. Evans, Reg. No. 26,269; Gary R. Edwards, Reg. No. 31,824, and Jeffrey D. Sanok, Reg. No. 32,169, to prosecute and transact all business in the Patent and Trademark Office connected with this application and any related United States and international applications. Please direct all communications to:

Evenson, McKeown, Edwards & Lenahan
1200 G Street, N.W., Suite 700
Washington, D.C. 20005
Telephone: (202) 628-8800
Facsimile: (202) 628-8844

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

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May 2 1995
Date

Signature

DECLARATION AND POWER OF ATTORNEY

Attorney Docket No. 1064/41979

Page 2

2 av

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Citizenship: Sweden

Postal Address/Residence: Sundbyberg, Sweden

SEX

May 2, 1995
Date

Birgitta Olofsson
Signature

...duced amino acid sequence of VEGF-B (Seq.1 and Seq.2)

```
                                                                    CG
  1                                                                 
    Gly Arg Pro Val Val Pro Trp Ile Asp Val Tyr Ala Arg Ala Thr Cys Gln   17
  3 GGA CGC CCA GTG GTG CCA TGG ATA GAC GTT TAT GCA CGT GCC ACA TGC CAG
    Pro Arg Glu Val Val Val Pro Leu Ser Met Glu Leu Met Gly Asn Val Val   34
 55 CCC AGG GAG GTG GTG GTG CCT CTG AGC ATG GAA CTC ATG GGC AAT GTG GTC
    Lys Gln Leu Val Pro Ser Cys Val Thr Val Gln Arg Cys Gly Gly Cys Cys   51
106 AAA CAA CTA GTG CCC AGC TGT GTG ACT GTG CAG CGC TGT GGT GGC TGC TGC
    Pro Asp Asp Gly Leu Glu Cys Val Pro Thr Gly Gln His Gln Val Arg Met   68
157 CCT GAC GAT GGC CTG GAA TGT GTG CCC ACT GGG CAA CAC CAA GTC CGA ATG
    Gln Ile Leu Met Ile Gln Tyr Pro Ser Ser Gln Leu Gly Glu Met Ser Leu   85
208 CAG ATC CTC ATG ATC CAG TAC CCG AGC AGT CAG CTG GGG GAG ATG TCC CTG
    Glu Glu His Ser Gln Cys Glu Cys Arg Pro Lys Lys Lys Arg Arg Val Leu  102
259 GAA GAA CAC AGC CAA TGT GAA TGC AGA CCA AAA AAA AAA AGG AGA GTG CTG
    Stop                                                            102
310 TGA AGCCAGACAGCCCCAGGATCCTCTGCCCGCCTTGCACCCAGCGCCGTCAACGCCCTGACCCCC
376 GGACCTGCCGCTGCCGCTGCAGACGCCCCCGCTTCCTCCATTGCCAAGGGCGGGGCTTAGAGCTCAA
443 CCCAGACACCTGTAGGTGCCGGAAGCCGCGAAAGTGACAAGCTGCTTTCCAGACTCCACGGGCCCGG
510 CTGCTTTTATGGCCCTGCTTCACAGGGACGAAGAGTGGAGCACAGGCAAACCTCCTCAGTCTGGGAG
577 GTCACTGCCCCAGGACCTGGACCTTTTAGAGAGCCTCTCTCGCCATCTTTTATCTCCCAGAGCTGCCA
644 TCTAACAATTGTCAAGGAACCTCATGTCTCACCTCAGGGGCCAGGGTACTCTCTCACTTAACCACCC
711 TGGTCAAGTGAGCATCTTCTGCCTGGGTGTCTCCCCTCACTATGAAAACCCCAAACTTCTACCAATA
778 ACGGGATTTGGGTTCTGTTATGATAACTGTGACACACACACACACTCACACTCTGATAAAAGAGAAC
845 TCTGATAAAAGAGATGGAAGACATAAAAAAAAAAAAAAAAAA
```

Figure 1

Deduced amino acid sequence of VEGF-B (Seq.2 and Seq.3)

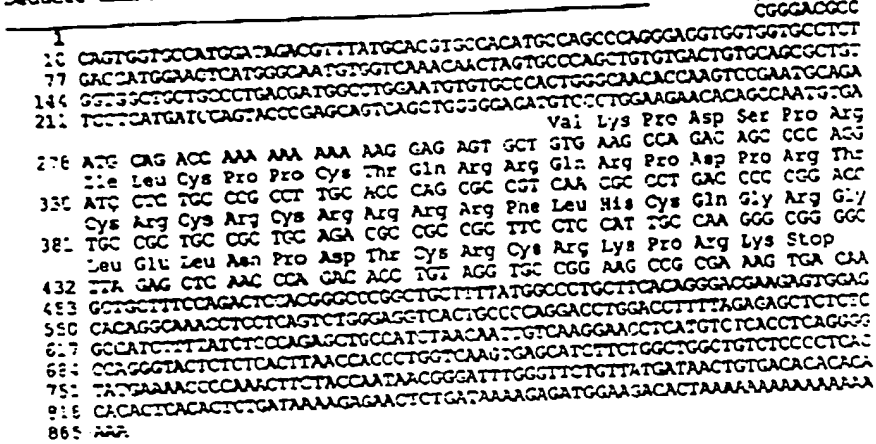

Figure 2

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Amino acid sequence alignments of PDGFs, PlGF, VEGF and the novel homologue VEGF-B

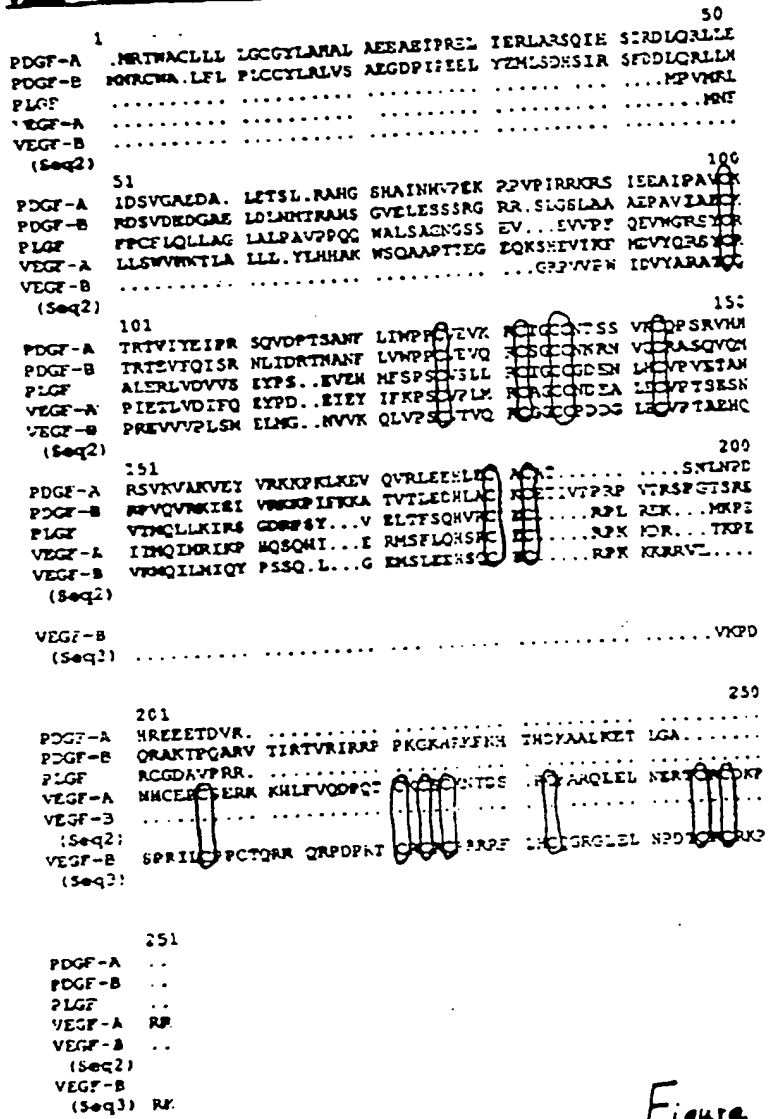

Figure 3